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RESEARCH ARTICLE

Chemical Profiling and Antihyperglycaemic Study on Butanol Fraction of *Chlorophytum alismifolium* Baker (Liliaceae)

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ABSTRACT

Purpose: Diabetes mellitus is a disorder associated with debilitating complications. This study was aimed at evaluating the chemical profile and antihyperglycaemic effect of butanol fraction of *Chlorophytum alismifolium*. **Methodology:** The powdered plant was extracted sequentially using soxhlet apparatus with solvents of varying polarities until butanol fraction was obtained. GC-MS analysis, phytochemical screening and acute toxicity studies were carried out. Antihyperglycaemic study was carried out using alloxan-induced hyperglycaemia in rats. Male Wistar rats were injected with 120 mg/kg of alloxan intraperitoneally, the rats with fasting blood glucose levels between 200 and 350 mg/dL were considered hyperglycaemic. Experimental groups were set up using normal rats in group I and hyperglycaemic rats in five groups of six rats each. Group II was the hyperglycaemic control while groups III, IV and V received the butanol fraction of *C. alismifolium* at 250, 500 and 1000 mg/kg respectively. Group VI received glimepiride 1 mg/kg. Blood glucose levels were monitored before treatment at 0 hour and 1, 2, 3 and 5 hours after treatment. **Findings:** Phytochemical screening revealed the presence of alkaloids, saponins, flavonoids, glycosides and triterpenes while GC-MS analysis revealed the presence of thirteen compounds some of which include; isoxazolidine, isothiazole and acetamide. Oral median lethal dose of the extract in rats was estimated to be >5,000 mg/kg. The butanol fraction of *C. alismifolium* at all the doses tested showed significant ($p<0.05$) blood glucose lowering effect when compared over time. **Conclusion:** The findings from this research showed that butanol fraction of *Chlorophytum alismifolium* possesses important compounds with antihyperglycaemic activity.

Keywords: Chlorophytum alismifolium; Hyperglycaemia; Gas chromatography-mass spectrometry

INTRODUCTION

Diabetes mellitus (DM) is a complicated metabolic disorder of the endocrine system which affects about 8.8 % of the global population[1]. The hallmark of type 1 diabetes is selective beta (β) cells destruction and severe or absolute insulin deficiency while type 2 diabetes is a heterogeneous group of conditions characterized by tissue resistance to the action of insulin combined with a relative deficiency in insulin secretion[2]. Chronic hyperglycaemia causes glycation of body proteins which lead to secondary complications[3]. Metabolic acute complications include; diabetic ketoacidosis and hyperosmolar non-ketotic coma while systemic late complications include; microangiopathy, diabetic nephropathy, diabetic neuropathy, diabetic retinopathy and cardiovascular diseases[4]. Due to a higher incidence of the risk factors, the prevalence of DM is increasing worldwide, but more evidently in developing countries[5] and the chronic complications resulting from diabetes mellitus are responsible for the majority of diabetes-related morbidity and mortality worldwide[6]. Globally, people living with diabetes were reported to be 425 million; Africa (15.9 million), Europe (58 million), Middle East and North Africa (38.7 million), North America and Caribbean (45.9 million), South and Central America (26 million), South and East Asia (82 million) and Western Pacific (158.8 million) and this alarming figures are projected to rise to a total of 629 million by the year 2045[1]. Insulin is used in the management of type 1 DM while other classes of drugs are used for type 2 DM and they include; sulphonylureas, biguanides, meglitinides, alpha glucosidase inhibitors, thiazolidinediones, dipeptidyl-peptidase-4 inhibitors, amylin analogues, incretin mimetics,

sodium-glucose transporter inhibitors, aldose reductase inhibitors and dopamine receptor agonists[2,7,8].

The chronic intake of orthodox drugs, the cost of acquiring them and their side effects have led people to resort to alternative therapy[9] and a significant percentage of the global population use medicinal plants for the management of DM and its complications[10]. One of such plants which is widely used by the people of Northern Nigeria is *Chlorophytum alismifolium*. It belongs to the family liliaceae and commonly known as Alimsa-leaved ground lili. The local names include; Hausa- *Rogon makwarwa*, Fulfulde-*Cigorodi* and Agatu- *Ekuce*. The tubers are used in the management of DM, pain and inflammatory conditions[11–13].

Gas chromatography-mass spectrometry is a standard technique with a broad range of applications in many areas of research including pharmaceutical and drug analysis[14]. It is also used in the identification and profiling of secondary metabolites found in natural products[15]. This study is aimed at establishing the chemical profile and evaluating the antihyperglycaemic effect of the butanol fraction of *C. alismifolium*.

MATERIALS AND METHODS

Materials

Alloxan (250316, Chem Light Laboratories, India), 10% Dextrose (Dana Pharmaceuticals, Nigeria), Glimepiride (Sanofi Aventis, France) and Normal saline (Dana Pharmaceuticals, Nigeria), Glucometer and test strips (Accu-check Active, Roche, Germany).

Experimental animals

Male Wistar rats (150-200 g) obtained from the Animal House, Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria were used for this study. The animals were maintained in a well-ventilated room, fed on standard feed and granted access to water *ad libitium*.

Preparation of butanol fraction of C. alismifolium

The whole plant of *Chlorophytum alismifolium* was collected from Tilden Fulani River in Toro Local Government Area of Bauchi state, Nigeria in June, 2018. It was identified and authenticated by Mallam Musa Muhammed of the Herbarium unit of the Department of Botany, Ahmadu Bello University Zaria, Nigeria. The plant was issued a voucher specimen number (No. 6785) for future reference.

The roots (tubers) were washed and chopped into smaller sizes and then air dried under shade for five weeks. The dried plant was then crushed into fine powder using pestle and mortar. The powdered plant (1 kg) was extracted sequentially with solvents of varying polarities, starting with hexane followed by ethylacetate and then methanol extract was obtained which was then partitioned in butanol to obtain the final fraction. The extract was concentrated to dryness on a water bath set at 45°C and then stored in a desiccator until further use. The preliminary phytochemical screening of the butanol fraction of *C. alismifolium* was carried out according to the methods of Evans[16].

Chemical profiling using gas chromatography-mass spectrometry

GC-MS analysis was performed using an Agilent 7890B GC system, 5977A mass spectrum detector (MSD) (Agilent Technologies, USA). The chromatography was performed on a HP-5 MS capillary column (30m×250μm×0.25μm). The carrier gas used was high purity helium and the con- stant flow rate of the helium was 3.6839 mL/min. Split injection ratio was 5:1. The temperature of the GC started at 50°C for 1 min, raised to 200°C at a rate of 3°C/min and then raised to 300°C at 3°C/min for 15 min and then held at 325°C (1 min). MS program scanned quality range of 30amu - 600amu, ionization voltage of 70eV, ionization current of 150μA (EI). The ion source and the quadrupole temperatures were set at 230°C and 150°C respectively. Compounds in the extract were identified on the basis of standards, isolation and structural determination in National Institute of Standards and Technology (NIST) 14. L database[17].

Acute toxicity study

The median lethal dose (LD50) of the extract was determined using the method described by Lorke[18]. The study was carried out in two phases: In the initial phase, three groups of three rats each were orally administered the extract of *Chlorophytum alismifolium* in widely differing doses of 10, 100 and 1000 mg/kg body weight and observed for signs of toxicity and mortality for 24 hours. In the second phase, three rats were orally administered the butanol fraction at the doses of 1600, 2900 and 5000 mg/kg body weight respectively and then observed for signs of toxicity post-administration and mortality after 24 hours after which the LD50 was estimated.

Alloxan-induced hyperglycaemia

The method described by Cooperstein and Watkins[19] was employed in overnight fasted rats. Forty five (45) Wistar rats were injected with alloxan monohydrate dissolved in sterile 0.9% normal saline and a dose of 120 mg/kg bw i.p was administered. The rats were then kept for the next 24 hours on 10% glucose solution since alloxan is capable of producing initial fatal hypoglycaemia. Three days post-induction with alloxan, the rats were monitored for hyperglycaemia using a glucometer (Accucheck Active, Roche Diagnostics, Germany). Rats with fasting blood glucose levels between 200 and 350 mg/dL were considered hyperglycaemic and selected for the study.

Experimental design

The rats (thirty alloxan-induced and six normal) were randomly divided into six groups; Group 1 served as the negative control and received the vehicle only (normal saline, 1 mL/kg). Group 2 served as the hyperglycaemic control which also received normal saline (1 mL/kg). Groups 3, 4 and 5 received graded doses of the butanol fraction of *C. alismifolium* at (250, 500 and 1000 mg/kg) respectively. Group 6 served as the positive control and received glimepiride (1 mg/kg b.w.). Blood samples were drawn from the tail vein prior to treatment at (0 h) and then at 1, 2, 3 and 5h after treatment. Fasting blood glucose levels were measured using the glucose-oxidase method.

Statistical analysis

Data of antihyperglycaemic study were expressed as Mean ± Standard Error of the Mean (S.E.M.) and the differences between means were analyzed by Repeated Measure Analysis of Variance (ANOVA) followed by Bonferroni post hoc test using a computer software application package (SPSS, Version 20). Values of $p<0.05$ were considered statistically significant.

RESULTS

Percentage yield and phytochemical constituents

The percentage yield of the butanol fraction of *Chlorophytum alismifolium* was calculated to be 2.49 % w/w and the phytochemical screening revealed the presence of alkaloids, saponins, triterpenes, glycosides, cardiac glycosides, tannins and flavonoids (Table 1).

Table 1: Phytochemical constituents of butanol fraction of *Chlorophytum alismifolium*

Constituents	Inference
Anthraquinones	–
Glycosides	+
Cardiac glycosides	+
Saponins	+
Flavonoids	+
Alkaloids	+
Triterpenes	+
Steroids	–
Tannins	+

Key: Absent – Present +

Chemical profiling

The GC-MS revealed the presence of thirteen compounds covering the total area of 100.2 % (Table 2).

Table 2: Chemical profile of butanol fraction of *C. alismifolium* using GC-MS

S/NO	Compounds	Area covered (%)	Retention time (min)
1	1-Methoxycyclohexane	4.8	5.33
2	N-Buthyl ether	13.21	5.56
3	1,3-Hexanediol	1.32	5.84
4	2-Propanone, Oxime	23.79	6.04
5	Cis-1-Butene	19.94	6.51
6	1-Propanone	4.9	9.8
7	1,10-Undecadiene	0.98	10.37
8	Acetamide	2.17	12.75
9	Isoxazolidine	11.93	15.1
10	Isothiazole	8.23	22.81
11	6-Methyl-triazolo-triazine	4.1	31.25
12	N-Ethylformamide	0.92	31.58
13	Propanamide	3.9	36.04

Median lethal dose

Oral administration of butanol fraction of *C. alismifolium* (10-5,000 mg/kg) did not produce any visible sign of toxicity or mortality in the animals over a period of 24 hrs. The oral LD_{50} was estimated to be above 5,000 mg/kg.

Effect of butanol fraction of C. alismifolium on alloxan-induced hyperglycaemic rats

A significant ($p<0.001$) increase in blood glucose levels were observed in the hyperglycaemic control following the administration of alloxan when compared to the normal control. Administration of the butanol fraction at all the doses tested (250, 500 and 1000 mg/kg) reduced the blood glucose levels when compared to the hyperglycaemic control, though the reduction wasn't statistically significant ($p>0.05$). The results were also compared over time by comparing 0 hour with the 1st, 2nd, 3rd, and 5th hours. The extract at 250 mg/kg significantly ($p<0.05$ and $p<0.001$) reduced the blood glucose level in the 3rd, and 5th hours respectively when compared to 0 hour. At 500 mg/kg, a significant ($p<0.01$ and $p<0.001$) reduction in blood glucose levels in the 3rd and 5th hours respectively were also observed when compared to 0 hour. At 1000 mg/kg, the extract significantly ($p<0.05$, $p<0.001$ and $p<0.001$) lowered the blood glucose level in the 2nd, 3rd, and 5th hours respectively when compared to 0 hour. (Figure 1).

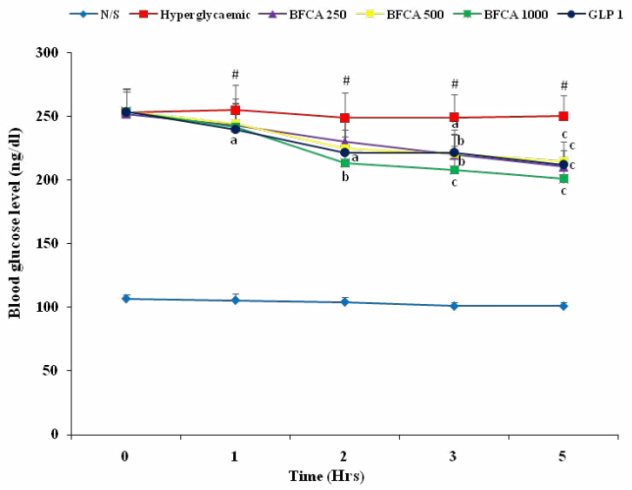

Fig. 1: Effect of butanol fraction of *Chlorophytum alismifolium* on blood glucose levels of alloxan-induced hyperglycaemic rats (Values Mean ± S.E.M., #=p<0.001 compared to N/S group, a =p<0.05, b =p<0.01, c =p<0.001 compared to 0 hr – Repeated measure ANOVA followed by Bonferroni post hoc test, n = 6, N/S = Normal saline, H/C = Hyperglycaemic control, BFCA = Butanol fraction of *Chlorophytum alismifolium*, GLP = Glimepiride)

DISCUSSION

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia with an increased risk of many complications[20] and herbal medicines are used for treatment of diabetes in developing countries[21]. In this study, the median lethal dose of the butanol fraction of *C. alismifolium* was > 5000 mg/kg implying that it is practically non-toxic when used orally.

Alloxan is a diabetogenic agent which can impair the activity of pancreatic β- cells and trigger hyperglycaemia[22,23]. In this study, administration of alloxan caused hyperglycaemia in the rats and the butanol fraction of *C. alismifolium* at the tested doses significantly reduced the fasting blood glucose levels in the hyperglycaemic rats. The phytochemical screening of the butanol fraction of *C. alismifolium* revealed the presence of secondary metabolites, some of which have been reported to have antihyperglycaemic activity. Several studies have linked phytochemicals like; flavonoids, alkaloids, triterpenes and saponins to antihyperglycaemic activity[24,25]. The phytochemical screening showed the presence of some of the aforementioned constituents which could probably be responsible for the observed antihyperglycaemic activity of the butanol fraction of *C. alismifolium*. The genus chlorophytum have been reported to be rich in biologically active saponins[26] which are phytochemicals that elicit their antihyperglycaemic activity through the restoration of insulin response and improvement in insulin signalling[27], increase in plasma insulin levels and stimulation of insulin release from the pancreas[28], insulin sensitization and antihyperlipidemic effect[29].

GC-MS system is valuable in the identification of the bioactive constituents of herbal medicines[30]. The chemical profiling of butanol fraction of *C. alismifolium* through GC-MS revealed the presence of some compounds with antihyperglycaemic activity. Hyperglycaemia especially in type 2 diabetes mellitus is not only caused by impaired insulin secretion from the pancreas but also by the increased insulin resistance in the peripheral tissues[31]. Hence, a decrease of insulin resistance is necessary for achieving normoglycaemia and isoxazolidine, one of the compounds found in the butanol fraction of *C. alismifolium* elicits its antihyperglycaemic activity by decreasing insulin resistance or improving insulin sensitivity in the target tissues[32]. Isothiazole is also one of the compounds found in the butanol fraction of *C. alismifolium* and its derivatives have been reported to act through the selective inhibition of aldose reductase, an enzyme in the polyol pathway which catalyzes the formation of sorbitol and thereby reducing some diabetic complications[33]. Synergism of these compounds with other phytochemical constituents may be attributed to the observed antihyperglycaemic activity of the butanol fraction of *C. alismifolium*.

CONCLUSION

The butanol fraction of *Chlorophytum alismifolium* contains bioactive compounds with potential blood glucose lowering effect and this justifies its use in the management of diabetes mellitus.

CONFLICT OF INTEREST

The authors have no conflict of interest with regards to this publication.

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